Muratech laboratory reports on the Upper Ottawa Street Landfill Site
Reference 21

MUTATECH INC.

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REPORT: AMES TESTS ON GASES EMANATING FROM VENTS IN THE UPPER OTTAWA STREET LANDFILL.

September, 1982.

P.O. BOX 198 KLEINBURG, ONTARIO, CANADA L0J 1C0

SUMMARY

Gases emanating from several vents in the Upper Ottawa Street Landfill have been analysed for mutagenicity using various modifacations of the Ames assay. The study began as an attempt to establish which of 3 possible methods would prove the most effective for the detection and quantitation of mutagenic activity. In the absence of any positive effect from any vent with any method it has been impossible to assess the relative effectivenes of the 3 techniques.

INTRODUCTION

The Ames assay (Ames etal, 1975) was developed for testing liquids or solids in solution and its use in testing vapours is an area which has not been thoroughly investigated. Modification of the assay to this end raises several problems in the laboratory but many more in the field where experimental conditions cannot be rigorously controlled.

This report provides details of analyses of gases emanating from vents on the Upper Ottawa Street Landfill for mutagenicity by various modifications of the Ames assay which have been designated the "bag", " desiccator" and "bubbling" techniques. The work was to be a comparison of the 3 methods and was carried out in accordance with protocols recommended by Mutatech and approved by the Study Committee. (Appendix I and II).

MATERIALS AND METHODS

Mutagenic activity was assayed using histidine auxotrophs of S. typhimurium Strains TA98, TA100, TA1535 and TA1537 were kindly provided by Dr. Bruce Ames, Berkeley, Calif., and were stored as frozen stocks at -70° C. The characteristics of these stocks are checked periodically (Histidine requirement, rfa character, R-Factor, uvr B deletion. Appendix III).

Bacterial cultures for all assays described were established by scraping a sterile wooden applicator stick cross the surface of the frozen stock, inoculating 5ml nutrient broth and shaking overnight at 37 degrees C.

"Bag" method a) vent 2.

2 ml Aliquots of top agar containing 0.1 ml of an overnight culture of strain TA100 or TA98 and 0.1 ml ampicillin solution (8 mg/ml in 0.02N NaOH) were poured onto petridishes containing approx 30 ml minimal agar. Four plates/ strain were then attached to 8" X 16" glass shelves using double backed adhesive tape. Tape was also used to secure the lids to the glass shelves which were then inverted and stacked in a polystyrene cooler box on and under ice bags.

Control plates were prepared similarly and stacked inverted on ice in a separate box.

Some 3 hrs later, after transport to the site, dishes were taken out of the coolers. Lids were removed and the glass shelves, with plates on the underside, arranged on aluminum shelf tracking attached to a wooden base (Fig. I). The whole fitted snugly inside a cardboard box, lined with black polythene, open at both ends and with a port at the top through which shelves could be removed at intervals. Surrounding and securely taped to the cardboard box was a large black garbage bag. A hole was cut into the top of the bag and the edges taped to the lid of the box. The mouth was gathered with an elastic band round the neck of one of 2 glass filler funnels taped end to end, containing loosely packed sterile cotton dressing. A short length of tygon tubing provided a connection between the funnels and the vent, the mouth of which was sealed off. A second length of tubing punched through the bag into the back of the apparatus and sealed with tape allowed suction to be drawn on the vent with a vacuum pump drawing at a rate of 5.4 L/min.

After an initial period of 10 min., shelves were removed at various intervals up to 4 hrs. Lids were replaced on dishes and secured with tape and the shelves

again stacked, inverted and placed on ice in cooler boxes. The sampling port was rapidly resealed with tape. Ambient temp at the start of the experiment was 21.5° C. This increased slightly during the course of the experiment - but the increase could not be measured after the thermometer was accidently broken.

Control plates, lids not removed and kept in black garbage bags, inside a cardboard box approx. 30 ft. away from the vent were returned to ice at intervals. Dishes were incubated at 37° C upon returing to the laboratory, some 2 hours after the final sample had been taken. At this time 3 plates were each exposed to a drop of MNNG (25 µg/ml, TA100)or 4NQO (5 µg/ml, TA98) to serve as positive controls. Colonies were counted 42 hours after the start of incubation.

b) Vents 2, 3 and 4, "gooseneck" pipe.

The gases emanating from the above vents were analysed in a similar manner to the above except that each apparatus held only 3 shelves and these were supported by pegboard hooks. Plates were prepared, transported to and from the site and incubated similarly. Vacuum pumps were used on vents 2 and 4 but not on vent 3 or the "gooseneck" pipe because of defective D.C. batteries. Accordingly, the cotton wool filters and funnels, which may have offered some resistance to the flow of gas into the apparatus, were omitted and the neck of the garbage bag taped round the mouth of each vent.

<u>"Desiccator" method:</u> vent 2 and 3

Agar plates were prepared as described above for each of strains TA98, TA100, TA1535 and TA1537. Ampicillin was omitted. On half of the plates 0.2 ml S9 mix

(Salamone et al, 1979)was incorporated into the top agar. Plates were arranged (inside a laminar flow hood) in 8L glass desiccators (Fig II), inverted without lids on sterile aluminum gauze spacers. Dishes were displaced vertically from one another to allow free flow of vapour through the stacks of plates.

Dessicators were sealed and placed inside black plastic bags. They were then put into tight fitting cardboard boxes lined with ½ inch polystyrene sheeting packed with ice bags. The lids of the boxes were closed so that only a short piece of tygon tubing containing a cotton plug leading to the vacuum port was exposed. The desiccators were then transported to the site. About 6 hours after sealing, they were partially evacuated using a hand pump to 12 or 7 in. Hg pressure, corresponding to about 50 and 25% evacuation, respectively.

Vacuum was maintained using a clamp and connecting it to a length of latex tubing leading one foot down the vent. The clamp was then released and after atmospheric pressure had been restored, each desiccator was resealed. During this period and during transportation back to the laboratory, the desiccators were never removed from ice. Upon returning to the laboratory approximately 2 hours later, the desiccators were placed in a hot room at 37°C, in the dark.

The desiccator containing control plates was evacuated to 12 in. Hg and opened in the laboratory. Several plates containing TA98 and no S9 mix were each exposed to a drop of 4NQO (5µg/ml) and others with S9 to 2-AA (100µg/ml).

"Bubbling Method": vent 3

Two 7.5 ml suspensions from overnight cultures of each of TA1537, TA98 and TA100 were placed on ice in a polystyrene cooler and transported to the site. They were then placed in a water bath at 37°C and gas emanating from the vent bubbled through one culture of each strain, using a multi-channel peristaltic pump a rate of approximately 1.5 cc/min. Gases were drawn through a cotton wool plug and the culture tubes capped and covered with aluminum foil during exposure.

At intervals up to 140 minutes, 0.5 ml. samples were removed from each of the tubes, both exposed and non-exposed, pipetted into culture tubes and placed on ice. On returning to the laboratory 2 hours later, 0.1 ml from each culture was added to 2.0 ml of molten agar, containing 0.1 ml of ampicillin (8mg/ml) in the case of TA98 and TA100, and Ames plates were poured as normal. 0.1 ml aliquots, taken at 0 time were added to top agar containing 0.1 ml 4NQO (5µg/ml, TA98), MNNG (25 µg/ml,TA100) or 9-AA (100 µg/ml TA1537) to serve as positive controls. Incubation was for 48 hours at 37°C.

RESULTS

These results constitute what was intended as the first phase of a two phase study, ie. to determine which of three possible alternatives was the best method for detecting and quantifying the mutagenic activity of gases emanating from the Upper Ottawa Street Landfill site.
Vent 2 was chosen to carry out the initial study because of data (Sciex Report to Upper Ottawa St. Landfill Site Study, Feb. 1982) indicating

that this vent was the richest in terms of the numbers of compounds identified in the gas phase. The bag method using TA98 and TA100 was used on two occasions at this site, and the desiccator method with TA1537, 1535, 100 and 98 on one occasion. No evidence of mutagenicity (by the usual criterea of at least 2X background reversion frequency and some indication of a dose related effect) was obtained in any of these experiments (Tables I, II and III). Furthermore, examination of the background lawn of bacteria exposed at this site gave no evidence of gross cell killing. Although cells were exposed in desiccators both in the presence and absence of S9 mix, it is likely that the liver enzymes were inactive at the time of exposure, since treatment of the plates with 2-AA did not give an increase in frequency above controls.

Three other locations were tested with the bag method: vents 3 and 4, and one of the several "gooseneck" pipes near Stonechurch Road. Again, no evidence of mutagenicity was found at any site (Table II). Lower colony numbers on exposed plates may be indicative of toxicity, this however was not reflected in the appearance of the background lawn.

Vent 3, by far the worst smelling vent on the landfill was tested using the desiccator and bubbling methods (Table IV). No positive mutagenic effect was observed with either technique.

In each of the various methods, fungal contamination of plates was observed. For reasons which are unclear, contamination was less of a problem on plates carrying TA100 than those with TA98. Contamination

was worse with the bag technique, than with the desiccator or bubbling methods. Bacterial infection could be prevented in the case of plates with TA98 and TA100, by incorporation of ampicillin into the top agar. This was used in all experiments except the desiccator method. In this case agar plates poured without cells, carried no contaminating bacteria, although fungal colonies were observed. Bacterial contamination was however clearly a problem in the bubbling method with TA1537. Infecting bacteria were identified by morphology and staining properties in the presence of crystal violet. It was not considered advisable to include these plates in our assessment of mutagenicity using the bubbling technique.

CONCLUSION

We conclude that as no positive response has been found at any vent with any method, it is not possible to assess the relative merits of the three techniques at this stage.

REFERENCES

AMES B.N., McCANN J., YAMASAKI E.: MUTATION RES.. 31 (1975) 347

SALAMONE M.F., HEDDLE J.A., KATZ M.: ENVIR. INT. 2 (1979) 37

ABBREVIATIONS

MNNG = N-methyl-N-nitronitrosoguanidine

9-AA = 9-aminoacridine

2-AA = 2-aminoflourine

4NQO = 4-nitroquinoline-N-oxide

TABLE I: BAG METHOD VENT 2

	TA98						TA100					
Treatment/Plate #	#1	#2	#3	#4	Mean	SEM	#1	#2	#3	#4	Mean	SEM
Control: ice	15	16	14		15.0	0.58	145	154	151		150.0	2.65
30min	12	14	9	12	11.75	1.03	206	195	194	197	198.0	2.74
60min	23	13	5	4			180	215	187	183		
	19 (#5)				12.8	3.75	202 (#5)				193.4	6.59
180min	8	16	8	13	11.25	1.97	174	145	132	144	148.75	8.92
4-NQO					+ve*							
MNNG											+ve*	
Treated: 15min	26	16	14	11	16.75	3.25	173	179	149	118	154.75	13.86
30min	11	18	22	17	17.0	2.27	155	179	148	152	158.5	6.98
45min	23	18	14	19	18.5	1.85	214	146	131	163	163.5	18.06
60min	14	11	11	20	14.0	2.12	224	150	193	167	183.5	16.14
90min	23	21	19	27	22.5	1.71	137	161	124	181	150.75	12.66
120min	20	9	14	21	16.0	2.8	143	119	196	144	150.5	16.23
180min	15	15	8	18	14.0	2.12	157	138	126	128	137.25	7.09
240min	2	16	18	16	13.0	3.7	199	204	152	196	187.75	12.03

* Clear positive response obtained after 'spot' test.

TABLE II: BAG METHOD VENTS 2,3&4, "GOOSENECK" PIPE.

	TA98						TA100					
Treatment/Plate #	#1	#2	#3	#4	Mean	SEM	#1	#2	#3	#4	Mean	SEM
Control: Ice	24	18	28	18	22.0	2.44	174	166	165	235	185.0	16.79
60min	24	17	20		20.33	2.03	230	180	172		194.0	18.15
4-NQO					+ve							
MNNG											+ve	
Vent 2: 40min	15	9	22	16	15.5	2.66	128	138	112	-	126.0	7.57
110min	10	11	12	-	11.0	0.58	147	145	118	135	136.25	6.63
135min	21	15	-	-	18.0	3.0	109	127	-	-	118.0	9.0
Vent 3: 70min	13	26	22	-	20.33	3.84	156	142	137	-	145.0	5.69
145min	-	-	-	-	-		144	-	-	-	144.0	
Vent 4: 85min	28	-	-	-	28.0		128	-	-	-	128.0	
150min	27	16	8	18	17.25	3.9	128	131	142	-	133.67	4.26
190min	11	26	19	12	17.0	3.49	129	160	110	123	130.5	10.6
"Gooseneck" pipe 55min	5	11	15	-	10.33	2.91	94	101	97	102	98.5	1.85
115min	10	7	-	-	8.5	1.5	106	146	98	127	119.25	10.81
155min	8	17	20	19	16.0	2.74	128	143	134	128	133.3	3.54

Missing plate counts mean either plate dropped off underside of glass shelf during exposure or fungal contamination too extensive.

TABLE III: DESICCATOR METHOD VENTS 2&3.

	- S9					+ S9				
Treatment/Plate #	#1	#2	#3	Mean	SEM	#1	#2	#3	Mean	SEM
TA98: Control	14	18	19	17.0	1.53	33	27	33	31.0	2.0
4-NQO				+ve						
2-AA						33	36	24	31.0	3.61
Vent 2, 7in Hg	11	14	14	13.0	1.0	33	25	22	26.67	3.28
Vent 2,14in Hg	11	14	11	12.0	1.0	23	24	34	27.0	3.51
Vent 3,14in Hg	20	22	12	18.0	3.06	18	25	-	21.5	3.5
TA100: Control	225	229	198	217.33	9.74	268	252	231	250.33	10.71
Vent 2, 7in Hg	209	172	170	183.67	12.68	230	245	259	244.67	8.37
Vent 2,14in Hg	241	244	240	241.67	1.2	265	267	235	255.67	10.35
Vent 3,14in Hg	268	216	225	236.33	16.0	302	309	317	309.33	4.33

TABLE III: DESICCATOR METHOD CONT.

	-S9					+S9				
Treatment/Plate #	#1	#2	#3	Mean	SEM	#1	#2	#3	Mean	SEM
TA1535: Control	30	28	22	26.67	2.4	30	21	-	25.5	4.5
Vent 2, 7in Hg	22	37	33	30.67	4.48	32	22	19	24.33	3.93
Vent 2,14in Hg	42	30	-	36.0	6.0	31	38	28	32.33	2.96
Vent 3,14in Hg	40	35	37	37.33	1.45	27	23	29	26.33	1.76
TA1537: Control	6	3	-	4.5	1.5	11	12	-	11.5	0.5
Vent 2, 7in Hg	3	5	2	3.33	0.88	10	12	12	11.33	0.67
Vent 2,14in Hg	6	5	9	6.67	1.2	10	15	11	12.0	1.53
Vent 3,14in Hg	5	6	-	5.5	0.5	6	6	5	5.67	0.33

TABLE IV: BUBBLING METHOD VENT 3

	Exposed					Unexposed				
Treatment/Plate #	#1	#2	#3	Mean	SEM	#1	#2	#3	Mean	SEM
TA 98 Cmin						17	11	13	13.67	1.76
25min	14	10	10	11.33	1.33	20	13	13	15.33	2.33
40min	12	9	7	9.33	1.45	17	15	12	14.67	1.45
60min	21	17	10	16.0	3.21	13	19	16	16.0	1.73
90min	17	13	18	16.0	1.53	18	19	24	20.33	1.86
120min	12	10	17	13.0	2.08	7	18	16	13.67	3.38
140min	7	15	10	10.67	2.33	10	9	11	10.0	0.58
TA100 Omin						113	101	93	102.33	5.81
25min	141	133	129	134.33	3.53	123	102	95	106.67	8.41
40min	160	118	118	132.0	14.0	97	111	107	105.0	4.16
60min	113	101	102	105.33	3.84	129	127	130	128.67	0.88
90min	128	109	80	105.67	13.96	116	121	90	109.0	9.61
120min	96	137	-	116.5	20.5	125	141	146	137.33	6.33
140min	120	118	95	111.0	8.02	123	135	130	129.33	3.48
TA98, 4-NQO(.5ug/plate)	297	420	-	358.5	61.5					
TA100,MNNG(25ug/plate)	Too high to count			+ve						

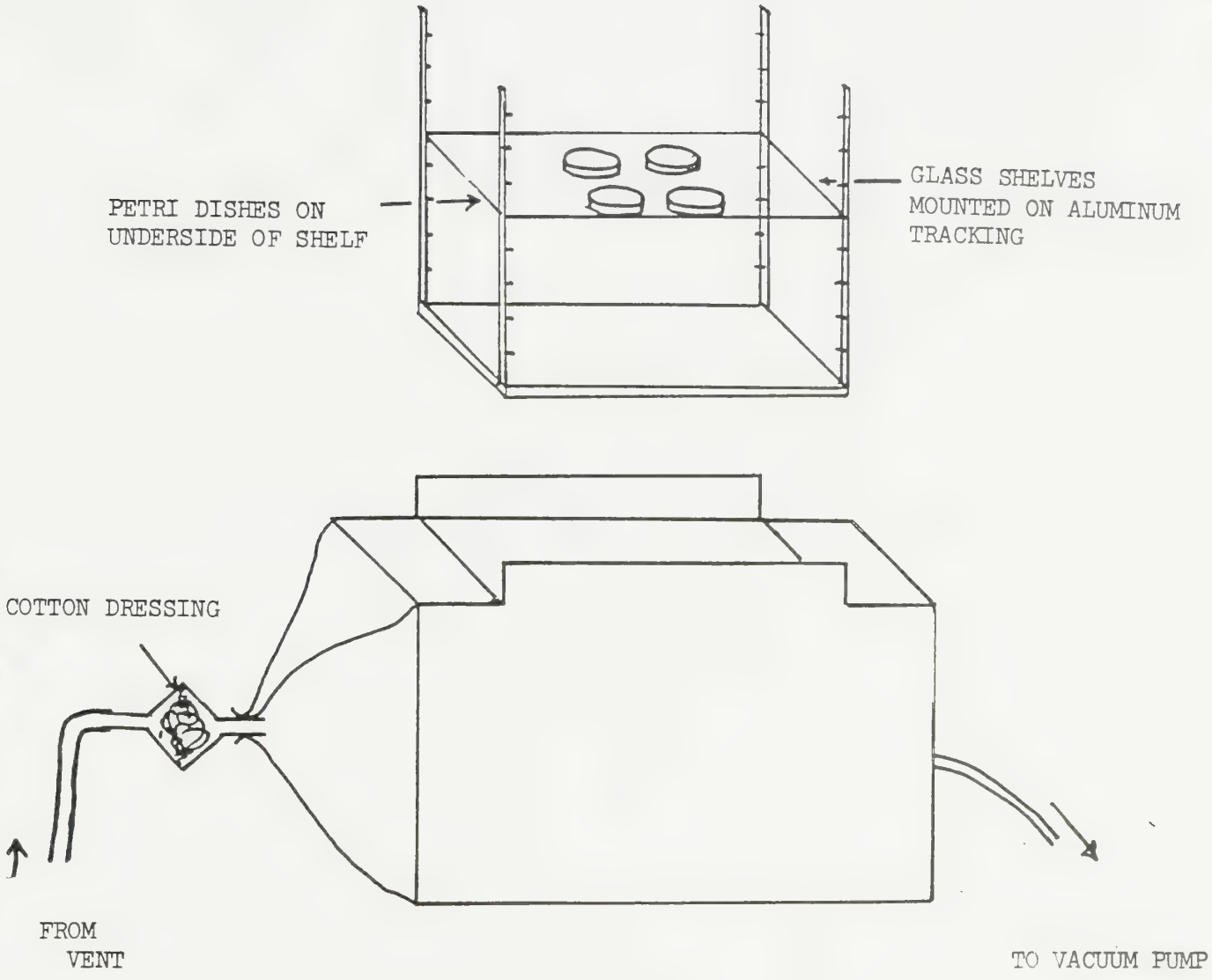

FIGURE I : BAG METHOD

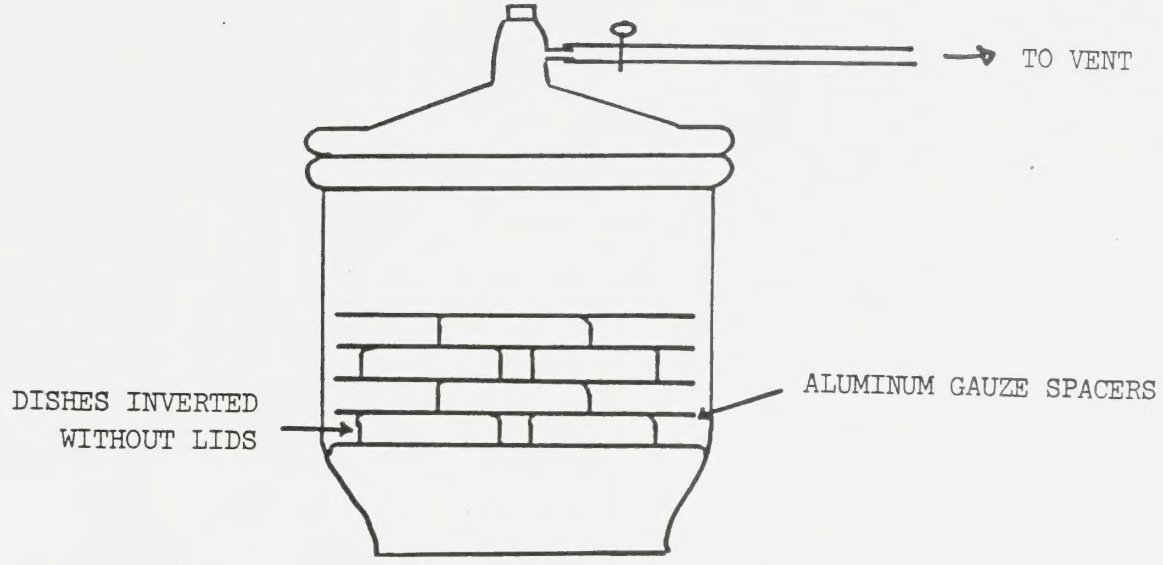

FIGURE II: DESICCATOR METHOD

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